Inhalation Toxicology, 2010; 22(3): 224–233

informa
healthcare

RESEARCH ARTICLE

Characterization of a head-only aerosol exposure system for nonhuman primates

P. A. Dabisch[1], J. Kline[1], C. Lewis[1], J. Yeager[2], and M. L. M. Pitt[1]

[1]Center for Aerobiological Sciences, United States Army Medical Research Institute of Infectious Disease, Fort Detrick, Maryland, USA, and [2]Akimeka, Inc., Frederick, Maryland, USA

Abstract

A well-characterized exposure chamber is necessary to generate reproducible atmospheres for inhalation toxicology studies. The aim of the present study was to characterize a head-only exposure chamber for non-human primates. Aerosols containing bovine serum albumin (BSA) were used to characterize a 16-L dynamic airflow head-only exposure chamber. A 250-ml plastic bottle with a respirator attached located inside the chamber was used to simulate a breathing head. Chamber leak rate, mixing, and aerosol spatial distributions were quantified. The chamber concentration profile was measured at the chamber exhaust using an aerodynamic particle sizer. Aerosol spatial distribution was determined by collecting filter samples at several chamber locations. The particle size distribution was determined by collecting cascade impactor samples at several chamber locations. The estimated chamber leak rate was within standards suggested in the literature. The measured average aerosol residence time was similar to theoretical aerosol residence time, suggesting that the chamber was mixing well. Additionally, the average concentration measured at each of the sampling locations within the chamber was similar, and the within-run coefficients of variation (CV) across all sampling locations was similar to those reported in previously published studies, again suggesting that the aerosol concentration throughout the chamber was uniform. The particle size distribution was similar throughout the exposure chamber. Additionally, the BSA concentration and particle size distributions measured in the breathing zone of the simulated head were not significantly different from measurements made elsewhere in the chamber, suggesting that respiration does not affect the average aerosol concentration or particle size distribution at the mouth.

Keywords: *Aerosol; exposure chamber characterization; head-only; nonhuman primates; particle size distribution*

Introduction

The ability to reproducibly deliver a given dose of an aerosolized test substance to an animal via the inhalation route requires a well-characterized exposure chamber. Chamber leaks, mixing characteristics, and particle size distribution can all affect the stability and reproducibility of exposure atmospheres (Dorato & Wolff, 1991). Therefore, an inhalation exposure chamber should be sealed tightly to minimize leaking/dilution of the chamber atmosphere, be optimized to provide adequate mixing of the chamber atmosphere to ensure a uniform distribution of the test atmosphere, and be operated at adequate flow rates relative to chamber volume to achieve both a rapid rise to equilibrium concentration of the test substance and adequate air changes for the animal(s) present. Additionally, a number of safety concerns need to be considered in the design of an inhalation exposure chamber. A chamber should be operated at a slight negative pressure to protect laboratory workers from exposure due to leaks in the chamber (Valentine & Kennedy, 2007). In some laboratories involved in research utilizing infectious bioaerosols, chambers are required to operate inside a class 3 biological safety cabinet to provide an additional barrier between the aerosol and laboratory personnel. However, this also places constraints on the size and capacity of the chamber.

It is difficult to construct an exposure chamber that is perfectly sealed. Thus, a number of different standards for exposure chamber leak rate have been proposed. O'Shaughnessy et al. (2003) recommended a fractional leak rate of 0.001 min^{-1} at −1 inch H_2O as a standard for exposure chambers. Cheng and Moss (1995) proposed that a chamber leak rate of less than 2% of the total chamber airflow is acceptable. Both criteria require that only a very

Address for Correspondence: Dr. Paul Dabisch, PhD, US Army Medical Research Institute of Infectious Diseases, Center for Aerobiological Sciences, 1425 Porter Street, Fort Detrick, MD 21702, USA. E-mail: paul.a.dabisch@us.army.mil

(Received 22 May 2009; revised 12 July 2009; accepted 16 July 2009)

ISSN 0895-8378 print/ISSN 1091-7691 online © 2010 Informa UK Ltd
DOI: 10.3109/08958370903191023

Report Documentation Page

Form Approved
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
12 JUL 2009		**00-00-2009 to 00-00-2009**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Characterization of a head-only aerosol exposure system for nonhuman primates	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Army Medical Research Institute of Infectious Disease,Center for Aerobiological Sciences,1425 Porter Street,Fort Detrick,MD,21702**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
see report

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**10**	

small fraction of the total chamber flow should be due to leaks, and that the vast majority of the chamber flow should be from sources built into the chamber design.

Chamber mixing can be characterized in several ways. The theoretical growth of aerosol concentration inside an exposure chamber can be described by the equation:

$$C = C_{SS} \cdot (1 - e^{-kt}) \quad (1)$$

where C is the theoretical concentration in the exposure chamber at a time, t ; C_{ss} is the theoretical steady state concentration; and k is the ratio of the chamber flow (Q_{cham}) to chamber volume (V_{cham}) (Moss, 1993; Pauluhn & Theil, 2007). The inverse of k is equal to the average residence time, τ, of an aerosol in the chamber. In a perfectly mixed chamber, the measured residence time, τ_{meas}, will be equal to the theoretical residence time, τ_{theo}. Thus, the degree of mixing of an exposure chamber can be estimated by determining how closely τ_{meas} matches τ_{theo} (O'Shaughnessy et al., 2003). A second method for estimating the degree of mixing in an exposure chamber involves measurement of the concentration of a test substance in the chamber at several discrete locations throughout the exposure chamber (Valentine & Kennedy, 2007). The variability in these measurements is inversely related to the degree of chamber mixing. Previous chamber characterization studies have reported coefficients of variation (CV) across sampling locations ranging from 4.8% to 15% (O'Shaughnessy et al., 2003; Lin et al., 2009). In addition to concentration measurements, measurement of the particle size distribution for atmospheres containing aerosolized test material is important as particle size is one of the major determinants of respiratory tract deposition. Ideally, a uniform concentration and particle size distribution should be present throughout the chamber. However, if the chamber atmosphere is not uniform, measurement of the chamber concentration and particle size distribution in the breathing zone of the exposed animal is sufficient to calculate an estimate of the inhaled dose (Environment Directorate, Organisation for Economic Co-operation and Development, 2008).

An inhalation exposure chamber should be operated at adequate flow rates relative to chamber volume in order to achieve a rapid rise to equilibrium concentration of the test substance. The t_{99} is defined as the time needed to reach a concentration of the test substance in the chamber equal to 99% of the steady-state concentration, and is equal to 4.605 times the ratio of V_{cham} to Q_{cham}. Minimizing the t_{99} increases the duration of time that the animal is exposed to the steady-state concentration, resulting in more consistent dosing throughout the exposure period. This is especially important for shorter duration exposures.

In the present study, a head-only exposure chamber for anesthetized nonhuman primates (NHPs) was characterized. The size of the chamber and the number of animals that could be exposed at a time were limited by available space as the system was designed to operate inside a class 3 biological safety cabinet. Thus, the chamber design was 16-L in volume and only able to accommodate a single NHP at a time. The use of a head-only exposure system minimizes the amount of aerosolized material deposited on the fur, thereby minimizing the dose received by the exposed animal due to routes other than inhalation (Dorato & Wolff, 1991). Some type of restraint, in this case chemical restraint in the form of anesthesia, is also required to ensure that the animal's head remains in the test atmosphere throughout the exposure period. Because the chamber is only able to expose a single animal at a time, the ability to reproducibly generate a given concentration of test substance in the chamber is critically important. A well-characterized chamber provides adequate information regarding chamber concentration and particle size distribution so that the inhaled dose can be accurately estimated. Chamber characterization included chamber leak testing, assessment of chamber mixing, and aerosol concentration and particle size spatial uniformity tests. Two different chamber configurations were tested—an empty chamber and a chamber with a simulated breathing head present—to determine if the presence of a breathing head altered airflow patterns and mixing in the chamber. Additionally, the feasibility of using respiratory-induced fluctuations in chamber pressure to determine respiratory parameters, namely tidal volume and respiratory period, was explored. Measurement of tidal volume and respiratory period in real time during an exposure would allow the delivered dose to be calculated on a breath-to-breath basis, resulting in more accurate dosing.

Disclaimer: animal use

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals, and adheres to the principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Methods

Exposure system configuration

The present study utilized an exposure system consisting of a head-only exposure chamber attached to an automated system for the control of chamber airflow, chamber pressure, aerosol generation, and aerosol samplers. The exposure chamber was constructed of lexan with internal measurements of 203 mm wide by 193 mm deep by 396 mm high. Airflow entered the chamber from a stainless steel tube with slotted sides, which entered the top of the chamber, and exited through a similar tube at the bottom of the chamber (Figure 1A). A 559 mm long stainless steel mixing tube (34 mm in diameter) was attached to the chamber inlet. A three-jet Collison nebulizer was attached to the other end of the mixing tube. The volume of the fully assembled chamber (V_{cham}), including the mixing tube,

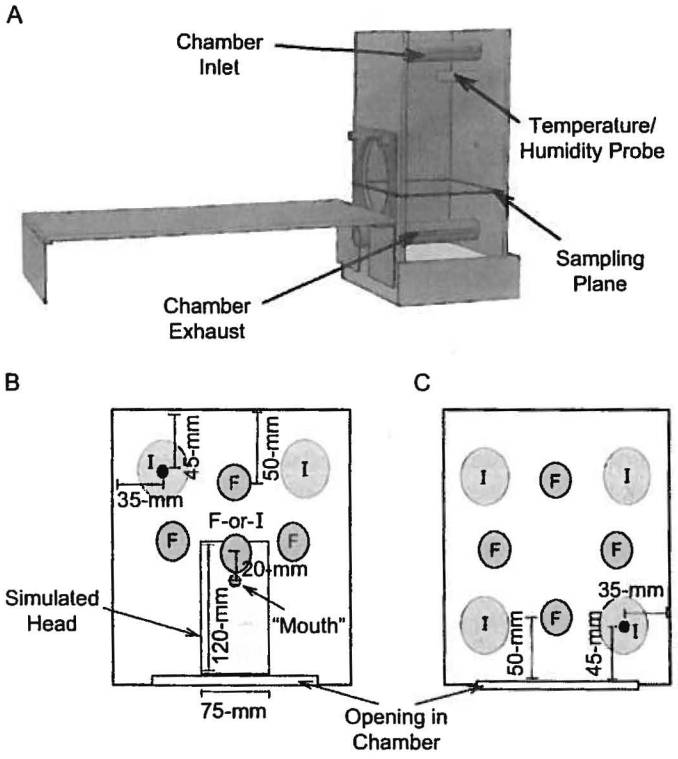

Figure 1. Exposure chamber diagram and sampling locations. (A) An anesthetized NHP is placed on the platform in a supine posture, with the head placed through an opening in the dental dam covering the round opening on the side of the chamber. The head is supported in the chamber by a wire mesh screen (not shown). The sampling plane is the plane in which the anesthetized animal's mouth is present during exposure. Airflow in the chamber is from top to bottom. (B) Sampling locations for filters (F) and cascade impactors (I) in the sampling plane of the chamber with a simulated breathing head present. (C) Sampling locations for filters (F) and cascade impactors (I) in the sampling plane of the empty chamber.

was 16.0 L. Normally, a round opening in one side of the chamber would allow the head of an anesthetized NHP to be inserted into the exposure chamber through a dental dam neck seal and exposed to the chamber atmosphere for a given period of time. In some portions of the present study, the round opening on the side of the chamber was covered with a piece of lexan to seal the chamber. Sampling ports located in the center of each wall of the chamber allowed samples of the chamber atmosphere to be collected from various locations inside the chamber. A graphic of the chamber with one side removed is shown in Figure 1A.

The automated control system was similar to a system described previously (Hartings & Roy, 2004), and consisted of an industrial automation controller (CFP-2010; National Instruments, Austin, TX) and associated I/O hardware (CFP-AI-110 and CFP-AO-210; National Instruments), a 0-30 SLPM flow controller to control the exhaust flow (MCR-100SLPM-D-30PSIA; Alicat Scientific, Tucson, AZ), a second 0-30 SLPM flow controller to control flow to the aerosol generator (MCR-30SLPM-D-.288; Alicat Scientific), a ±2.5-inch WC differential pressure transducer (sampling rate = 50 Hz; PX655-2.5BDI; Omega Engineering, Stamford, CT), a temperature and humidity sensor (HX94C; Omega Engineering), and an aerodynamic particle sizer (APS; Model 3321; TSI, Shoreview, MN) with a 1:20 aerosol diluter (Model 3302A; TSI) attached. The control hardware was enclosed separately and tethered via an Ethernet cable to a laptop PC running custom control software developed using LabView 8.5 with real-time component (National Instruments). The automation controller allowed the laptop to remotely monitor and/or control each flow controller set point; total chamber flow; chamber temperature, humidity, and pressure; and to collect and analyze the output of the APS.

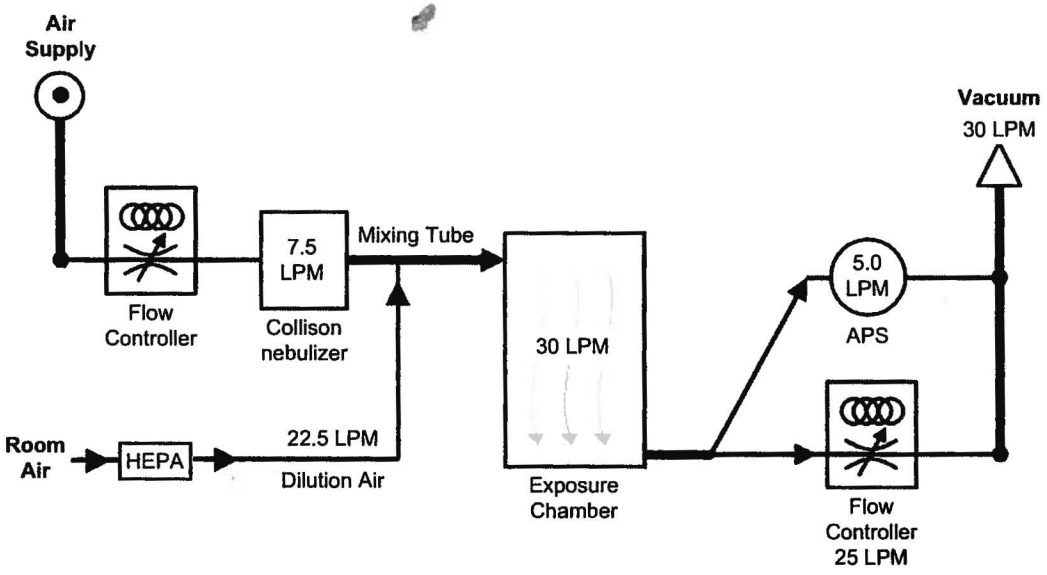

Figure 2. Control of airflow in the exposure chamber. Airflow in the chamber is determined by the exhaust flow, totaling 30 L/min between the APS and vacuum. Air is pulled into the chamber through a HEPA-filtered inlet and mixes with the output of the Collison nebulizer. Air flows through the chamber from top to bottom and is exhausted from the bottom.

The exposure chamber and control hardware were connected as depicted in Figure 2. The system was run in a 'pull' configuration with the exhaust flow controller set to 25 L/min (Moss, 1993). The APS was located on the exhaust side of the chamber and pulled 5 L/min, resulting in a total exhaust flow of 30 L/min. The volume of the tubing located between the chamber and the APS was 0.47 L (the common exhaust tubing was 0.29 L and the sampling tube from the exhaust bifurcation to the APS inlet was 0.18 L). The 30 L/min being pulled by the exhaust enters the chamber through a HEPA-filtered inlet open to room air just proximal to the exposure chamber from the Collison nebulizer. The aerosol generator used was a three-jet Collison nebulizer (BGI, Waltham, MA), which requires an air supply of 7.5 L/min. Because total chamber airflow was held constant at 30 L/min, only 22.5 L/min were drawn through the HEPA-filtered inlet when the Collison nebulizer was operating.

Chamber leak rate determination

To estimate the chamber leak rate, the chamber was sealed, and a 0-2 SLPM flow controller (MC-2SLPM-D; Alicat Scientific) was connected to the chamber through a sampling port in the side of the chamber. A lexan plate was placed over the opening in the chamber where the NHP head would normally enter the chamber. The flow controller was set to a range of different volumetric flows (0.00–0.40 L/min) and the corresponding chamber pressure for each flow rate was recorded. Because all other chamber inlets and outlets were sealed, the flow pulled by the flow controller was equivalent to the chamber leak rate at the measured chamber pressure. These data were used to construct a graph of leak rate versus chamber pressure, and linear regression was used to generate a line equation to fit the data. Thus, for any given chamber operating pressure, the chamber leak rate could be estimated. It should be noted that the leak rate determined using this method does not include any leaks that may be present around the dental dam neck seal that is used when an NHP is present in the chamber.

Chamber leak rate was also estimated in an operating chamber with a lexan plate sealing the opening in the side of the chamber. Total chamber flow (Q_{cham}) was set to 30 L/min, and the airflow at the chamber inlet was measured using Gilibrator-2 Diagnostic Calibration System (Sensidyne LP, Clearwater, FL). The chamber leak rate was equal to the difference between the total chamber flow, controlled by the exhaust flow controller, and the flow measured at the chamber inlet.

To determine the leakiness of the NHP neck seal, the chamber was also run with an anesthetized NHP present with a dental dam neck seal. Two African Green monkeys and two rhesus macaques anesthetized with telazol (2.5 mg/kg given intramuscularly [i.m.]) were used. Each NHP was placed in a supine position on a lexan platform, and the head was placed through the dental dam neck seal on the chamber. The NHP head was oriented so that the mouth was facing into the airflow of the chamber. Chamber pressure was recorded at 50 Hz for 10 min for each NHP.

Chamber mixing and spatial uniformity testing

To quantify chamber mixing and spatial uniformity, an aerosol atmosphere was generated in a system operating with Q_{cham} equal to 30 L/min. All of these runs utilized bovine serum albumin (BSA; A7511; Sigma-Aldrich, St. Louis, MO) dissolved in phosphate-buffered saline to a final concentration of 28 mg/ml and aerosolized using a Collison nebulizer. Two different chamber configurations were tested: (1) an empty chamber controlled by the automated control system described previously, and (2) a chamber controlled by the automated control system with a 250-ml bottle present in the position that a NHP head would be located during an actual exposure. The 250-ml bottle is approximately the size of the head of the NHPs commonly used. Tubing from a respirator (Inspira; Harvard Apparatus, Holliston, MA) located outside the exposure chamber passed through the interior of the bottle to a hole ("mouth"; Figure 1B) on the surface of the bottle, allowing the respiratory inhalation and exhalation pattern of a NHP to be simulated. The respirator was set to a tidal volume of 30 ml and a respiratory rate of 20 breaths per minute. These values were based upon historical data collected in our laboratory from anesthetized NHPs (data not shown).

In order to quantify chamber mixing, the average residence time, τ, of aerosolized BSA in the chamber was determined by measuring the concentration-time profile at the chamber exhaust. The theoretical residence time (τ_{theo}) of a chamber is equal to the ratio of V_{cham} to Q_{cham} (O'Shaughnessy et al., 2003). In a perfectly mixed chamber, the measured residence time (τ_{meas}) will be equal to the τ_{theo}. Thus, the degree of mixing of an exposure chamber can be estimated by determining how closely τ_{meas} matches τ_{theo}. If a chamber was not mixing ideally, as occurs in the presence of nonventilated dead space, the value of τ_{meas} would be less than the τ_{theo} because the apparent volume of the chamber is less than the actual physical volume of the chamber due to the presence of the dead space. τ_{meas} can be obtained by taking the inverse of k in Equation 1. The chamber concentration profile and particle size distribution was recorded every 5 s using the APS located on the exhaust for the duration of each 10-min run. Concentration versus time was plotted for each run and nonlinear regression was used to fit Equation 1 to the measured concentration data (SigmaPlot 11.0; Systat Software, San Jose, CA). τ_{meas} was calculated for each run of the chamber and the average value was compared to τ_{theo} to estimate the actual mixing performance of the chamber.

Chamber mixing was also assessed by determining the amount of aerosolized BSA collected on filter samples at four different locations in the chamber (Figure 1B and C). Aerosol samples were collected on 25-mm type A/E glass fiber filters in 25-mm in-line Delrin filter holders (Pall Life Sciences, East Hills, NY). Filter holders were

located 50 mm away from the center of each wall of the chamber in the same plane of the chamber that the head of the anesthetized NHP would be located during an actual exposure and attached to sampling ports located in the center of each wall of the chamber. When the simulated head was present, one of the filters was located in the breathing zone of the simulated head, 20 mm from the outlet of the respirator on the head (Figure 1B). The filters were operated at a flow of 50 ml/min using external 0-2 SLPM flow controllers (MC-2SLPM-D; Alicat Scientific). The flow used provided isokinetic sampling, assuming the presence of laminar flow at the filter inlet, so that the chamber atmosphere was minimally disturbed by the sampling devices. At the end of the 10-min exposure period, in each filter was removed from its filter holder and placed a 5-ml polystyrene round-bottom tube (Becton-Dickinson, Franklin Lakes, NJ). Two milliliters of phosphate-buffered saline were added, the tubes were vortexed for 10 s, and allowed to sit for an additional 10 min. The liquid from each tube was pippetted into a 1.5-ml Eppendorf tube (Westbury, NY) and centrifuged at 10,000 rpm for 10 min to remove any filter debris (5414C Centrifuge; Eppendorf). The supernatant was assayed for protein concentration using a BCA Protein Assay kit (Pierce Biotechnology, Rockford, IL) and a SpectraMax spectro-photometer (Molecular Devices, Sunnyvale, CA). Results were reported in micrograms of BSA per milliliter of supernatant. Recovery of known amounts of BSA spiked onto 25-mm glass fiber filters was found to be 80.3% ± 5.8% (CV = 7.2%; data not shown). Therefore, BSA values collected from the chamber were divided by 0.803 to account for the incomplete recovery. Average chamber aerosol concentration was calculated by taking the total amount of protein present in the 2 ml of filter supernatant and dividing it by the total volume of air that passed through the filter during the aerosol generation period.

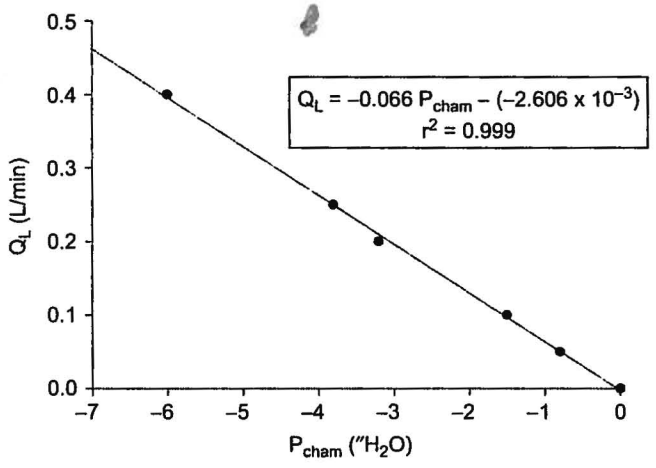

Figure 3. Chamber leak rate versus chamber pressure. A 0-2 SLPM flow controller was set to a range of different volumetric flows (0.050-0.400 L/min) and the corresponding chamber pressure for each flow rate was recorded. Because the chamber was sealed, the flow pulled by the flow controller was equivalent to the chamber leak (Q_L) rate at the measured chamber pressure (P_{cham}).

The particle size distribution of aerosolized BSA was determined using seven-stage stainless steel Mercer impactors (250 ml/min; In-tox Products, Moriarty, NM) with the impactor inlet located in the plane that the head of the anesthetized NHP would be located during an exposure. Impactors were located at one of five locations in the chamber (Figure 1B and C). The amount of BSA deposited on each impactor stage was determined using a BCA protein assay kit (Pierce Biotechnology). The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were determined as described previously (Environment Directorate, Organisation for Economic Co-operation and Development, 2008). Particle size data collected from the center of the exposure chamber with impactors was compared to particle size data collected using the APS located on the chamber exhaust.

Statistical analyses

All values presented are mean ± standard deviation. SigmaPlot 11.0 (Systat Software) was used for all statistical testing. Equation 1 was fitted to the data acquired using the APS using the nonlinear regression function (single, two-parameter exponential rise to maximum model). Concentration and particle size data were compared across different chamber configurations using unpaired *t* tests. An alpha value of .05 was used as the criterion for statistical significance.

Results

Chamber leak rate

Chamber leak rate was determined in a sealed chamber at a number of different pressures, and these results are shown in Figure 3. Linear regression yielded a line equation of $Q_L = -0.066\ P_{cham} - (-2.606 \times 10^{-3})$ ($r^2 = .999$), where Q_L is the chamber leak rate in L/min and P_{cham} is the chamber pressure in inch H_2O. With the aerosol generator operating as it would be during an exposure and the lexan plate covering the opening in the chamber, the chamber pressure was −0.509 ± 0.011 inch H_2O (CV = 2.1%; $n = 7$). Based on the results of the linear regression analysis, the estimated leak rate at this operating pressure was 0.036 L/min, or 0.12% of the total chamber airflow, with a calculated fractional leak rate (chamber leak rate/chamber volume) equal to 0.0023 min^{-1}. At a chamber operating pressure of −1 inch H_2O the estimated leak rate was 0.066 L/min, resulting in a fractional leak rate of 0.0043 min^{-1}.

When the chamber was operated with the exhaust flow set to 30 L/min with the lexan plate sealing the opening in the chamber, the airflow measured at the chamber inlet using a Gilibrator flow meter (Sensidyne LP) was 29.79 ± 0.88 L/min ($n = 10$; CV = 2.97%).

With both the aerosol generator operating and an anesthetized NHP present in the chamber with a dental dam seal around the neck, the chamber pressure was −0.448 ± 0.031 inch H_2O (CV = 6.9%; $n = 4$), or 88% ± 6%

(range = 96.3–83%) of the pressure attained when the opening in the side of the chamber was sealed with a lexan plate.

Chamber mixing and spatial uniformity

Operating the chamber at 30 L/min resulted in a theoretical t_{99} of 2.5 min. In an empty chamber, Q_{cham} was constant at 30 L/min, or 0.5 L/s, and V_{cham} was 16 L. An additional volume of 0.29 L was present in the exhaust tubing located between the chamber and the bifurcation to which the APS was attached. The flow in this section was 30 L/min. The sample tubing supplying the APS had a volume of 0.18 L and a flow of 5 L/min (0.083 L/s). The theoretical residence time, τ_{theo}, is equal to the sum of the values of each of these individual segments. In this case, the value of τ_{theo} was equal to 34.8 s. The value of k obtained using nonlinear regression to fit a curve to the growth-phase APS concentration data obtained from an empty chamber was 0.0317 ± 0.0062 s^{-1}, yielding a τ_{meas} of 32.6 ± 5.9 s (CV = 18.2%; n = 11). The average r^2 value of the fitted curves was .968 ± .022 (CV = 2.3%). A representative chamber concentration profile for an empty chamber and its associated fitted curve is shown in Figure 4A. Another series of chamber runs were conducted with a 250-ml bottle attached to a respirator placed in the chamber in the approximate location that an anesthetized NHP head would be located in an actual exposure to simulate a breathing NHP head. In this configuration, the Q_{cham} was constant at 30 L/min, or 0.5 L/s, and the V_{cham} was equal to 15.75 L. The volumes and flows for the exhaust and APS sample tubing were the same as stated previously. The value of τ_{theo} for this configuration was 34.2 s. The average value of k obtained using nonlinear regression to fit a curve to the growth phase concentration data obtained from a chamber with a simulated head present was 0.0338 ± 0.0060 s^{-1}, yielding a τ_{meas} of 30.5 ± 6.0 s (CV = 19.6%; n = 9). The average r^2 value of the fitted curves was .964 ± .027 (CV = 2.8%). A representative chamber concentration profile for a chamber with a simulated head present and its associated fitted curve is shown in Figure 4B.

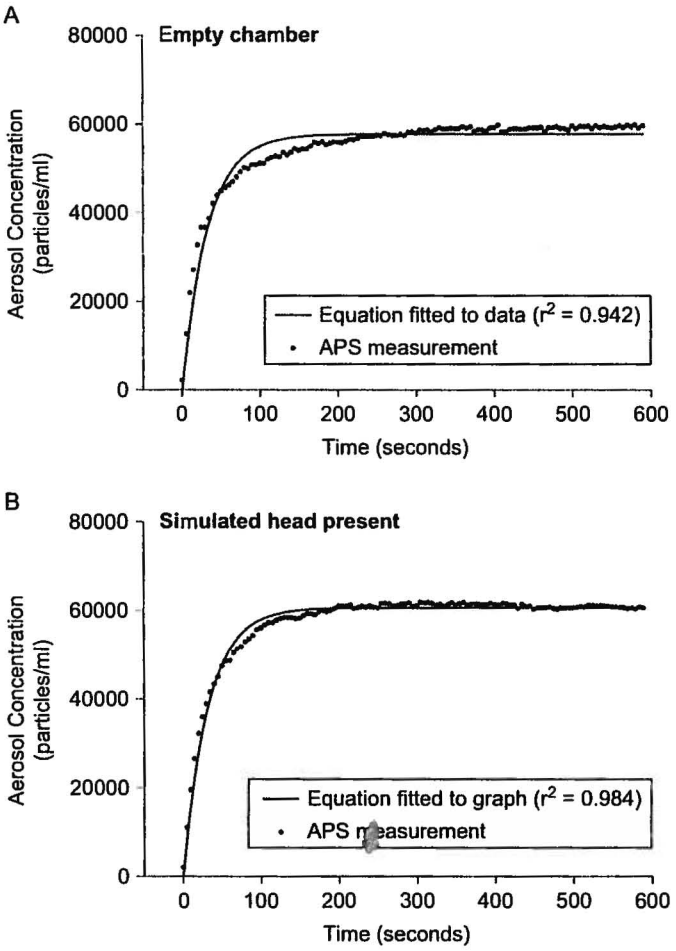

Figure 4. Representative chamber concentration profiles (as measured at the exhaust) for an empty chamber and a chamber with a simulated breathing head present. (A) For an empty chamber, fitting Equation 1 to the APS concentration data resulted in a value of k of 0.0303 s^{-1}, and a value of T'_m of 33.0 s (r^2 = .942). The theoretical values of T_{theo} was 34.8 s. (B) For a chamber with a simulated head present, fitting Equation 1 to the APS concentration data resulted in a value of k of .0309 s^{-1}, and a value of T'_m of 32.4 s (r^2 = .984). The theoretical values of T_{theo} was 34.2 s.

The average BSA concentration measured in the empty chamber was 61 ± 10 µg/L across six different runs of the chamber, resulting in a between-run coefficient of variation of 16.3%. The average within-run coefficient of variation across the four sampling locations was 9.6% ± 7.5% (Table 1). With a simulated head present, the average BSA concentration was 70 ± 10 µg/L across 13 different runs of the chamber, resulting in a between run coefficient of variation of 13.6%. This was not significantly different from chamber runs conducted without the simulated head present (Table 1). The average within run coefficient of variation between the four sampling locations was 10.0% ± 6.8%. This value was not significantly different from runs conducted without the simulated breathing head present. The average BSA concentration in the breathing zone of the simulated head was 69 ± 10 (CV = 14.8%; n = 6). This value was not significantly different from the average BSA concentration measured at the other three sampling locations in the chamber.

Table 1. Particle size and concentration data for an empty chamber and a chamber with a simulated head present.

	Particle size distribution			Concentration		
Chamber configuration	Parameter	Mercer impactor	APS	$[BSA]_{avg}$ (µg/L)	Between runs CV	Within runs CV
Empty	MMAD (µm)	1.76 ± 0.13	1.79 ± 0.19	61 ± 10	16.3%	9.6%
	GSD	2.09 ± 0.10	2.22 ± 0.25			
Simulated head present	MMAD (µm)	1.75 ± 0.15	1.74 ± 0.07	70 ± 10	13.6%	10.0%
	GSD	1.79 ± 0.16	2.15 ± 0.06			

The average MMAD measured using cascade impactors at four sampling locations in the empty chamber was 1.76±0.13 µm (CV = 7.7%) with a GSD of 2.09±0.10 (CV = 4.8%). Log-probability plots of the cumulative mass of BSA collected by the stages of the cascade impactor were linear (r^2 = .993 ± .009; CV = 0.9%), suggesting that the particle size distribution was lognormal (Thiel, 2002). The APS located on the chamber exhaust measured an average MMAD 1.79±0.19 µm (CV = 10.4%; n = 8) with a GSD of 2.22±0.25 (CV = 11.1%; n = 8) during the same runs of the chamber (Table 1). The particle size distributions obtained using the Mercer impactors located in the exposure chamber and the APS located on the chamber exhaust were not significantly different from each other. The average MMAD measured using cascade impactors located at three sampling locations with the breathing simulated head present was 1.75±0.15 µm (CV = 8.8%) with a GSD of 1.79±0.16 (CV = 9.0%). One of the three sampling locations was located in the breathing zone of the simulated head. The MMAD and GSD measured in the breathing zone were 1.79±0.18 µm and 1.83±0.19, respectively (n = 4), and were not significantly different from the values measured at the other two sampling locations (MMAD = 1.71±0.13 µm; GSD = 1.74±0.12; n = 5). Log-probability plots of the cumulative mass of BSA collected by the stages of the cascade impactor were linear (r^2 = .988 ± .008; CV = 0.8%), suggesting that the particle size distribution was lognormal (Theil, 2002). The APS located on the chamber exhaust measured an average MMAD 1.74±0.07 µm (CV = 4.1%; n = 6) with a GSD of 2.15±0.06 (CV = 2.7%; n = 6) for the same chamber configuration (Table 1). The MMAD was not significantly different from the value measured from the center of the chamber with cascade impactors. However, the GSD measured using the APS was slightly, but significantly, larger than the value measured with the cascade impactors (Table 1).

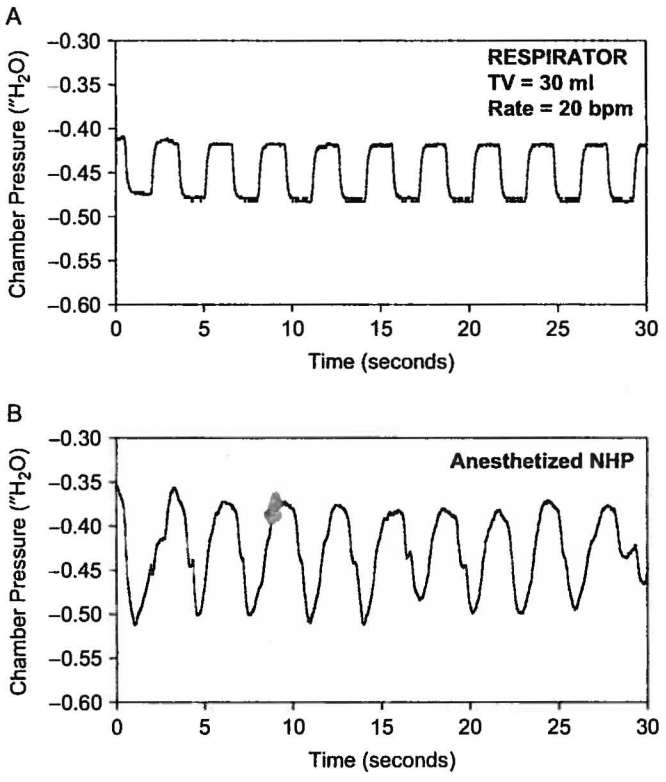

Figure 5. Representative chamber pressure tracings for a chamber with a simulated breathing head present and a chamber with an anesthetized NHP present. (A) Tracing from the chamber with the simulated head and a respirator set to a rate of 20 bpm and a tidal volume of 30 ml. Baseline chamber pressure was adjusted to −0.450 inch H_2O, the average baseline pressure measured with an anesthetized NHP and dental dam neck seal. (B) Tracing from the chamber an anesthetized NHP and dental dam neck seal. Given the magnitude of the changes in chamber pressure with the simulated and NHP head present, future iterations of the chamber will be able to incorporate real-time plethysmography based on the fluctuations in chamber pressure in order to calculate tidal volume and respiratory period in real time, thereby increasing the accuracy of dosing.

In a chamber with a simulated breathing head present with a tidal volume of 30 ml, a rate of 20 bpm, and a baseline pressure of −0.45 inch H_2O, the chamber pressure oscillated between approximately −0.400 to 0.500 inch H_2O and the oscillations were synchronized with the cycle of the respirator (Figure 5A). With an anesthetized NHP present, the baseline chamber pressure was approximately −0.448±0.031 inch H_2O, and the chamber pressure oscillated between approximately −0.350 to −0.550 inch H_2O due to the respiration of the animal (Figure 5B).

Discussion

The aim of the present study was to characterize a head-only dynamic airflow exposure chamber. The chamber is designed to operate inside a class 3 biological safety cabinet to expose anesthetized NHPs to aerosolized infectious agents for exposure periods of less than 20 min. The exposure system had a total chamber flow of 30 L/min. Chamber leaks and mixing affect the ability to reproducibly generate stable aerosol atmospheres (Dorato & Wolff, 1991). Thus, in the present study, chamber characterization included chamber leak testing, chamber mixing characterization, and aerosol concentration and particle size spatial uniformity tests.

A number of different standards for leak rates in exposure chambers have been proposed. O'Shaughnessy et al. (2003) suggested a fractional leak rate of 0.001 min^{-1} at −1 inch H_2O as a standard for exposure chambers, whereas Cheng and Moss (1995) suggested that a chamber leak rate of less than 2% of the total chamber airflow is acceptable. In a chamber sealed with a lexan plate, the leak rate was found to be between these two standards, with a leak rate equal to 0.12% of the total chamber flow at the operating pressure of −0.509 inch H_2O, and a fractional leak rate of 0.0043 min^{-1} at −1 inch H_2O. When this chamber configuration was operated under normal conditions with an exhaust flow set to 30 L/min using a calibrated flow controller, the measured inlet flow was 29.79±0.88 L/min, suggesting that the chamber leak rate is negligible under normal operating conditions. In a chamber

with an anesthetized NHP present and a dental neck seal, the operating pressure of the chamber, averaged over 10 min to minimize the influence respiratory fluctuations in chamber pressure, was -0.448 ± 0.031 inch H_2O. This value was significantly less negative than the chamber sealed with a lexan plate, suggesting that the dental dam seal around the neck is leaking more than the lexan plate. The range of chamber pressures with a dental dam neck seal ranged from -0.491 to -0.423 inch H_2O, compared to -0.509 inch H_2O with a lexan plate seal. Thus, the leakiness of the dental neck seal varies slightly from animal to animal, and therefore the effect of the leak in the neck seal on aerosol distribution and mixing will vary slightly from animal to animal. Additional testing is necessary to determine if the leakiness of the neck seal results in disturbances in aerosol distribution and mixing in the chamber. Alternatively, measurements of concentration and particle size made near the breathing zone of the animal would still be sufficient to calculate an estimate of the inhaled dose (Environment Directorate, Organisation for Economic Co-operation and Development, 2008).

For mixing and spatial variability tests, two different chamber configurations of the lexan-sealed chamber were tested: (1) an empty chamber controlled by the automated control system, and (2) a chamber controlled by the automated control system with a simulated breathing head present in the position that a NHP head would be located during an actual exposure to determine its effect on aerosol distribution in the chamber. In an empty chamber, the average measured aerosol residence time was 32.6 ± 5.9 s, nearly identical to the theoretical value of 34.8 s, suggesting that the empty chamber was mixing ideally under the operating conditions tested. The average BSA concentration was 61 ± 10 μg/L across six different runs of the chamber, resulting in a between-run coefficient of variation of 16.3%, suggesting good reproducibility of the chamber concentration between runs. The average within-run coefficient of variation between the four sampling locations was 9.6% ± 7.5%. This value was similar to previous published studies (O'Shaughnessy et al., 2003; Lin et al., 2009), suggesting the spatial distribution of aerosol within the chamber was similar to other exposure chambers.

In a chamber with a simulated head present, the average measured aerosol residence time was 30.5 ± 6.0 s, again similar to the theoretical value of 34.2 s. The average BSA concentration in the empty chamber was 70 ± 10 μg/L across 13 different runs of the chamber, resulting in a between-run coefficient of variation of 13.6%, suggesting good reproducibility of the chamber concentration between runs. The average BSA concentration obtained with a simulated head present was not significantly different from the value obtained from an empty chamber The average within-run coefficient of variation between the four sampling locations was 10.0% ± 6.8%. This value is again similar to previous published studies and was not significantly different from the value obtained from an empty chamber. The BSA concentration measured in the breathing zone of the simulated head was not significantly different from the concentrations measured at other sampling locations in the chamber, suggesting that the inspiratory and expiratory respiratory patterns do not significantly alter the average aerosol concentration present in the breathing zone. However, it should be noted that this chamber configuration does not utilize the leakier dental dam neck seal. Because the neck seal is near the breathing zone, it is possible that the leakier dental dam neck seal may produce changes in the aerosol concentration and particle size distribution in the breathing zone relative to other sampling locations in the chamber. However, as noted previously, measurements of concentration and particle size made near the breathing zone of the animal would still be sufficient to calculate an estimate of the inhaled dose (Environment Directorate, Organisation for Economic Co-operation and Development, 2008).

The particle size distribution of both configurations was also determined. In an empty chamber, the variability in the particle size distribution measurements from the four cascade impactor sampling locations in the chamber was less than 8%, suggesting that the particle size distribution was similar throughout the exposure chamber. Additionally, the particle size distribution obtained with cascade impactors was similar to the distribution obtained from the APS located on the chamber exhaust. In a chamber with the simulated head present, the MMAD measured using cascade impactors located in the breathing zone of the simulated head was not significantly different from the MMAD measured at peripheral locations in the chamber, from the APS located on the chamber exhaust, or from the empty chamber. However, the width of the particle size distribution measured in the center of the chamber using cascade impactors with the breathing simulated head present was slightly narrower than that measured using the APS at the chamber exhaust. Given that small differences may exist between particle size distributions measured from cascade impactors located in the center of the chamber and from an APS located on the chamber exhaust, it is advisable that measurements of the particle size distribution be made as close to the breathing zone as possible to ensure that an accurate measurement of the inhaled aerosol is obtained.

Taken together, the concentration and particle size data suggest that both the empty chamber and the chamber with the simulated head present are mixing well under the operating conditions tested. Additionally, the concentration and particle size distributions in the breathing zone of the chamber with the simulated head present are similar to those obtained elsewhere in the chamber, suggesting the expiratory and inspiratory respiratory pattern of the simulated head does not significantly alter the aerosol concentration and particle size distributions. Finally, the particle size distribution of aerosolized BSA measured in both chamber configurations is similar to the particle size distributions of bacteria and viruses commonly aerosolized using a three-jet Collison in our laboratory (data

not shown). Thus, the chamber characterization data using BSA is applicable to other agents with the same particle size distribution. However, for particle size distributions that differ significantly from that of BSA, spatial uniformity tests of the aerosol concentration and particle size distributions will need to be performed.

To conduct useful inhalation toxicology studies, it is necessary to be able to reproducibly deliver a given dose of a test substance to an animal. The dose of an aerosolized agent deposited in the respiratory tract of an exposed animal can be estimated by the equation:

$$D = \sum_{size=lo}^{hi} f_{size} \cdot C_{size} \cdot MV \cdot t \quad (2)$$

where the deposited dose (D) is equal to the sum of the individual doses for each particle size present in the particle size distribution. The minimum (size = lo) and maximum (size = hi) particle sizes are based on the measured particle size distribution. The individual dose for a given particle size is the product of the deposition fraction of a given particle size of the inhaled substance in the respiratory tract (f_{size}), the concentration of the given particle size of the aerosolized substance (C_{size}) in a homogenously distributed chamber (or the concentration of the given particle size of the aerosolized substance in the animal's breathing zone in a nonhomogenously distributed chamber), the respiratory minute volume (MV), and the duration of the exposure segment in minutes (t). A recent publication also suggests expressing the dose calculated using a calculation similar to the above calculation per unit of body weight (Alexander et al., 2008), and this additional calculation can easily be done post-exposure.

The first term in the dosing equation (Equation 2), the deposition fraction (f), is often assumed to be 100% in the absence of deposition data in order to represent a worst/best case scenario. In this case, Equation 2 simplifies to Equation 3, where C is the average concentration of the test substance in the chamber across all particle sizes. However, a significant amount data exist regarding deposition efficiency of different particle sizes in different regions of the respiratory tract of different species (Newton, 2002). Thus, other values for the deposition fraction can be justified, and, indeed, have been used in the literature (Alexander et al., 2008).

$$D = C \cdot MV \cdot t \quad (3)$$

Chamber concentration can be estimated at any time during the exposure period using Equation 1, assuming that the steady-state concentration of the test substance is known. The steady-state concentration can be determined by performing sham runs in which no animal is present. In the system characterized in the present study, there was no difference between the concentration profiles and particle size distributions obtained from an empty chamber and from a chamber with a simulated head present. Thus, sham runs conducted without an animal present could be used to estimate the steady-state concentration expected with a NHP present. If Equation 2 is being used, then the concentration of the test substance of a given particle size can be estimated by breaking up the estimated steady state concentration according to the measured or estimated particle size distribution.

Minute volume is often estimated using one of several equations based on body weight (Alexander et al., 2008; Bide et al., 2000; Guyton, 1947). Alternatively, minute volume can be measured directly. In past studies utilizing a similar exposure system to the one described in the present study, minute volume in anesthetized NHPs has been measured immediately before the exposure using head-out plethysmography, and the measured minute volume has been used as an estimate of the minute volume during the exposure period (Besch et al., 1996). However, these methods estimate respiratory parameters, and do not provide direct measurement of minute volume during the exposure period, potentially resulting in a decrease in the accuracy of the calculated delivered dose. The system characterized in the present study monitors chamber pressure at a sampling rate of 50 Hz. Based on the comparison of chamber pressures between an empty chamber, a chamber with the simulated breathing head present, and a chamber with an anesthetized NHP present, future iterations of the chamber will aim to incorporate real-time plethysmography based on the fluctuations in chamber pressure in order to determine respiratory parameters, specifically tidal volume, and respiratory period.

By determining respiratory period and tidal volume in real time and calculating the average chamber concentration of the test substance during each breath, the delivered dose can be calculated for each individual breath and the accumulated dose for the entire exposure period can be determined. This may enhance the accuracy of dosing because estimates of minute volume are no longer used in the calculation. However, this approach presents additional challenges relating to synchronization of the respiratory and chamber concentration calculations, and integration of these data into the automated exposure control system. However, the results of this study suggest that such a calculation is theoretically possible.

In summary, the present study characterized a dynamic airflow head-only exposure chamber for NHPs. The chamber was designed to expose NHPs to infectious bioaerosols, and therefore must be operated inside a class 3 biological safety cabinet, limiting the capacity of the chamber to a single NHP. The chamber leak rate was within suggested standards, the spatial distribution of aerosol throughout the chamber was of uniform concentration and size across the sampling locations, and this distribution was reproducible across discrete runs of the system. Additionally, the presence of a simulated breathing NHP head did not alter the chamber aerosol or particle size distributions, allowing runs with an empty chamber to be used to estimate the steady-state concentration of a particular

test substance. The dental dam neck seal used with NHPs is slightly, but significantly, leakier than the lexan plate used with the simulated head, and the influence of this leak on the aerosol concentration and particle size distribution is unknown. However, measurements made near the breathing zone of the animal will still allow an accurate estimation of the inhaled dose. Finally, based on the comparison of chamber pressures between an empty chamber and a chamber with the simulated breathing head present, future iterations of the chamber may be able to increase the accuracy of dosing by incorporating real-time plethysmography based on fluctuations in chamber pressure.

Declaration of interest

The work carried out for this study was funded by the US Defense Threat Reduction Agency.

References

Alexander DJ, Collins CJ, Coombe DW, Gilkison IS, Hardy CJ, Healy G, Karantabias G, Johnson N, Karlsson A, Kilgour JD, McDonald P. 2008. Association of Inhalation Toxicologists (AIT) Working Party Recommendation for Standard Delivered Dose Calculation and Expression in Non-Clinical Aerosol Inhalation Toxicology Studies with Pharmaceuticals. Inhal Toxicol 20:1179–1189.

Besch TK, Ruble DL, Gibbs PH, Pitt ML. 1996. Steady-state minute volume determination by body-only plethysmography in juvenile rhesus monkeys. Lab Anim Sci 46:539–544.

Bide RW, Armour SJ, Yee E. 2000. Allometric respiration/body mass data for animals to be used for estimates of inhalation toxicity to young adult humans. J Appl Toxicol 20:273–291.

Cheng YS, Moss OR. 1995. Inhalation exposure systems. In: RO McClellan and RF Henderson, eds. Concepts in Inhalation Toxicology. 2nd ed. Boca Raton, FL: CRC Press, 25–61.

Dorato MA, Wolff RK. 1991. Inhalation exposure technology, dosimetry, and regulatory issues. Toxicol Pathol 19:373–383.

Environment Directorate, Organisation for Economic Co-operation and Development. 2008. Draft Guidance on Acute Inhalation Toxicity Testing. OECD Environment, Health and Safety Publications, Series on Testing and Assessment. No. 39, 28 November 2008.

Guyton AC. 1947. Measurement of the respiratory volumes of laboratory animals. J Appl Physiol 150:70–77.

Hartings JM, Roy CJ. 2004. The automated bioaerosol exposure system: Preclinical platform development and a respiratory dosimetry application with nonhuman primates. Inhal Toxicol 49:39–55.

Lin WC, Hu S, Hartsell S, Wegner A, Juergensmeye M, Gingras B, Holland L. 2009. Characterization of an efficient nebulizer for generating bioaerosol exposures. In: Program of 7th American Society of Microbiology-Biodefense and Emergency Dissease Research Meeting. Abstract 09(K),p. 47.

Moss OR. 1993. Inhalation toxicology: Sampling techniques related to the control of exposure atmospheres. In: K Wileke, PA Baron, eds. Aerosol Measurement—Principles, Techniques, and Applications. New York: John Wiley and Sons, 833–842.

Newton PE. 2002. Fundamental inhalation toxicology. In: Derelanko, Hollinger, eds. Handbook of Toxicology. 2nd ed. Boca Raton, FL: CRC Press, 267–334.

O'Shaughnessy PT, Achutan C, O'Neill ME, Thorne PS. 2003. A small whole-body exposure chamber for laboratory use. Inhal Toxicol 15:251–263.

Pauluhn J, Thiel A. 2007. A simple approach to validation of directed-flow nose-only inhalation chambers. J Appl Toxicol 27:160–167.

Thiel CG. 2002. Cascade impactor data and the Lognormal distribution: Nonlinear regression for a better fit. J Aerosol Med 15:369–378.

Valentine R, Kennedy GL. 2007. Inhalation toxicology. In: Hayes AW, ed. Principles and Methods of Toxicology. 5th ed. Boca Raton, FL: CRC Press, 1407–1457.